killing and can be used to purge leukemic cells from hematopoietic stem cell (HSC) products in an experimental murine transplantation model. We now wish to extend our studies to examine the use of PS-341 administration post-HSC transplant. We hypothesized that PS-341 would reduce GVHD associated mortality by preventing degradation of IkB leading to decreased NF-kB DNA binding activity and NF-kB dependent cytokine production. We tested the efficacy of PS-341 administration in a full MHC mismatched murine model of acute lethal GVHD. C57BL/6 bone marrow and splenocytes were transplanted into lethally irradiated BALB/c hosts. PS-341 (1mg/kg) was administered on the day of transplant and also at later time points in some groups. Under experimental conditions where animals that receive vehicle control succumb to acute GVHD within 12 days of transplant, mice that received PS-341 on the day of transplant are protected from lethal disease (log rank test p< 0.0001). However, administration of PS-341 during the first week post-transplant resulted in markedly accelerated mortality due to GVHD. These studies demonstrate that the proteasome inhibitor PS-341 can be given concurrent with allogeneic HSC transplantation and can reduce early lethal acute GVHD although timing of the administration is critical.

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TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) RELAPSING AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH STI-571 (IMATINIB MESYLATE) WITH OR WITHOUT DONOR LYMPHOCYTE INFUSION (DLI)

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CML relapsing after allogeneic stem cell transplantation (SCT) can be successfully retreated with DLI. However DLI can cause GVHD and is less effective for hematological relapse. We therefore used STI-571 to treat patients with CML relapsing after SCT following myelobalative preparative regimens. Ten patients (9 who had not received STI-571 pretransplant) relapsed 4-36 months post transplant. Five were molecular relapses, five were more advanced (karyotypic =1, chronic phase (CP) = 2, blast crisis (BC) =2). STI-571 300-600 mg/day was given to all patients who had a minimum of two positive RT-PCR assays *2 months apart, or who developed hematological relapse. Prior to treatment, immunosuppression was stopped on all patients (n=9) without active GVHD. STI as a single agent was given to 5 molecular relapses. Two had failed prior DLI. Four patients promptly became PCR negative within 6 weeks. However three had a molecular relapse after STI was stopped and are currently retreated. STI-571 combined with 1-5 x107/kg CD3+ DLI was given to 5 patients relapsing with more advanced CML. Three (CP or karyotypic relapse) achieved complete molecular remissions, sustained in two. One myeloid BC patient developed extensive chronic GVHD after DLI given day 100 to treat persisting molecular disease, STI was stopped because of thrombocytopenia, he progressed with chloromas by 5 months post-transplant but has achieved a stable molecular remission persisting >6 months after restarting STI 600mg/day. One patient relapsing in ALL BC achieved a hematological remission but relapsed again 3 months later and died of disease progression.Thus, of 10 STI-treated patients, 7 achieved molecular remission which was sustained in 4. These results suggest that the cytoreductive action of STI can synergize with the graft-versus-leukemia effect. However, the tendency for further relapse when STI is withdrawn suggests that cure of relapsed CML may also require a GVL effect.

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INFUSION OF HIGH NUMBERS OF G-CSF MOBILIZED BLOOD DENDRITIC CELLS TYPE 2 (DC-2) IS ASSOCIATED WITH AN INCREASED RATE OF CHRONIC GVHD IN ALLOGENEIC PBSC TRANSPLANTATION

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It was previously shown that dendritic cells type-2 (DC-2) are significantly increased in G-CSF-mobilized leukapheresis products as compared to unstimulated bone marrow. In this study, we analysed whether the numbers of DC-1 and DC-2, as well as of other cell components in the graft, was associated with acute and/or chronic GVHD in 31 adult patients (11 MM, 7 CML-CP, 6 AML, 5 NHL, and 2 MDS) receiving an allogeneic PBSC transplant from HLA-matched siblings. Average cell doses (x106/kg) in the grafts were the following: 283±137 CD3+ T cells, 160±88 CD4+ T lymphocytes, 116±55 CD8+ T lymphocytes, 64±39 CD19+ B lymphocytes, 51±29 CD56+ NK cells, 253±103 CD14+ monocytes, 6.6±4.1 CD34+ cells, 2.1±0.9 HLA-DR+lin-CD11c+ DC-1 and 2.9±1.3 HLA-DR+lin-CD123+ DC-2. Median follow up was 255 days (range: 50-685). Patients were initially divided in three groups according to whether they had shown no signs of acute GVHD (grade 0, n=10), acute GVHD grade I (n=12), or grade II - IV (n=9). Median numbers of CD34+ cells, lymphocyte subsets, and DC-1 and DC-2 received by patients in these three groups did not differ significantly. Of 21 patients with adequate follow up (median 485 days, range: 131-695) 12 developed chronic GVHD (10 extensive, 2 limited). Analysis of cell components of the grafts demonstrated that patients developing chronic GVHD had received a significantly higher dose of DC-2 than patients without chronic GVHD (3.3±1.5 vs 2.2±0.9, p=0.05), while the dose of DC-1 (p=0.7), monocytes (p=0.28), T lymphocytes (p=0.643), B lymphocytes (p=0.939), NK cells (p=0.487) and CD34+ cells (p=0.757) was not different. Also, chronic GVHD did not correlate with recipient or donor age or gender, interval between diagnosis and transplant, presence of ATG or TBI in the conditioning regimen, or type of GVHD prophylaxis. Our results suggest that the presence of large DC-2 numbers in the graft may be associated with a higher risk of chronic GVHD after allogeneic PBSC transplantation. These data might prompt further studies addressing whether depletion of graft DC-2 might be beneficial in this setting.

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SOLID TUMOR VACCINES ELICIT DISTINCT IMMUNE RESPONSES FROM HOST VERSUS DONOR T CELLS IN MIXED CHIMERAS CREATED BY NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION (NST)

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A growing body of evidence suggests that functional unresponsiveness of the immune system to growing solid tumors may be due to tolerance in tumor-specific $CD4^+$ T cells, whereas tumor-specific $CD8^+$ T cells exist in a state of partial activation with limited effector function. We therefore hypothesized that an allogeneic graft-versus-host reaction may provide a helper effect that augments the anti-tumor immune response of host $CD8^+$ T cells in mixed hematopoietic chimeras. To test this hypothesis, we analyzed host versus donor T cell responses to AH1, an H-$2L^d$-restricted tumor antigen expressed by CT-26, a colon cancer of BALB/c mice. BALB/c mice with pre-established subcutaneous tumor underwent NST from MHC-compatible B10.D2 donors. Two weeks later, groups of chimeras received nothing, B10.D2 splenocytes IV, CT-26 tumor vaccine (irradiated CT-26 cells mixed with a GM-CSF secreting bystander cell line), or both. By staining with H-$2L^d$ tetramers loaded with the AH1 423-431 a.a. peptide, AH1-specific $CD8^+$ T cells were found to comprise 2-3% of total spleen $CD8^+$ T cells three weeks after vaccination. In contrast, AH1-specific T cells could not be detected in the non-transplanted, tumor bearing mice. To further characterize AH1-specific T cells we characterized T cell avidity for antigen, as measured by binding of AH1 peptide-loaded, L^d IgG dimmers. AH1 specific T cells were easily expanded in vitro using AH-1 peptide pulsed irradiated BALB/c splenocytes. After four weeks of stimulation the highest percentages of cultures containing AH1-specific T cells were derived from tumor-bearing chimeras that received B10.D2 splenocytes and a CT-26 vaccine. Expanded cultures contained AH-1 specific $CD8^+$ T cells derived from the host and the donor, based on the differential expression of Ly9.1 marker on the